Association between neutrophil to lymphocyte ratio and pulmonary arterial hypertension

Nötrofil/lenfosit oranı ile pulmoner arter hipertansiyonu arasındaki ilişki

Abdulkadir Yıldız, M.D., Hasan Kaya, M.D., Faruk Ertaş, M.D., Mustafa Oylumlu, M.D., Mehmet Zihni Bilik, M.D., Murat Yüksel, M.D., Nihat Polat, M.D., Mehmet Ata Akil, M.D., Zuhal Atılgan, M.D., Mehmet Sıddık Ülgen, M.D.

Department of Cardiology, Dicle University Faculty of Medicine, Diyarbakir

ABSTRACT

Objectives: Pulmonary hypertension (PH) is composed of a heterogeneous group of disorders marked by increased pulmonary artery resistance leading to right heart failure, with high mortality. Evidence is increasing to propose that inflammation plays a significant role in the pathophysiological mechanism. Increased prevalence of PH in patients with systemic inflammatory diseases is already known. Herein, we sought to evaluate the association between neutrophil to lymphocyte ratio (N/L ratio) and pulmonary arterial hypertension (PAH).

Study design: Twenty-five patients with PAH and 25 controls were evaluated. Baseline clinical and echocardiographic variables were obtained. Complete blood counts in all patients and controls were reviewed retrospectively.

Results: The N/L ratio was higher in patients with PAH compared to healthy volunteers (p=0.05). A cut-off value of 1.65 for N/L ratio predicted the presence of PAH with 72% sensitivity and 69% specificity. After multivariate analysis, only N/L ratio remained a significant predictor of PAH.

Conclusion: We showed for the first time that N/L ratio was significantly increased in patients with PAH compared to controls.

ÖZET

Amaç: Pulmoner hipertansiyon (PH) sağ ventrikül yetersizliği ve yüksek mortalite ile seyreden artmış pulmoner arter direnci ile kendini gösteren heterojen bir hastalık grubundan oluşur. Son zamanlarda patofizyolojik mekanizmada enflamasyonun merkezi bir rolü olduğu ile ilgili kanıtlar artmaktadır. Sistemik enflamatuvar hastalıklarda PH prevalansının artmış olduğu zaten bilinmektedir. Bu çalışmada, nötrofil/lenfosit oranı (N/L oranı) ile pulmoner arter hipertansiyonu (PAH) arasındaki ilişki değerlendirildi.

Çalışma planı: Pulmoner arter hipertansiyonu bulunan 25 hasta ve sağlıklı 25 gönüllüden oluşan kontrol grubu değerlendirildi. Bazal klinik ve ekokardiyografik bulgular kaydedildi. Tüm hasta ve kontrol grubunun tam kan sayımları geriye dönük olarak değerlendirildi.

Bulgular: Nötrofil/lenfosit oranı kontrol grubuna göre PAH'lı hastalarda daha yüksekti (p=0.05). N/L oranının 1.65 eşik değeri PAH varlığını %72 duyarlılık ve %69 özgüllük ile öngörmekteydi. Çok değişkenli analizden sonra sadece N/L oranının PAH'ın anlamlı öngördürücüsü olarak kaldığı saptandı.

Sonuç: Nötrofil/lenfosit oranının kontrol grubuna göre PAH'lı hastalarda daha yüksek olduğu gösterildi.

Pulmonary hypertension is a multifactorial, chronic and progressive disease causing right heart dysfunction and eventually death. Excessive vasoconstriction, thrombosis and abnormal vascular remodeling result in increased pulmonary vascular resistance and right ventricular afterload.[1,2]

While pulmonary arterial hypertension (PAH) was once regarded mainly as a disease of excessive vasoconstriction, it is now considered to be a vasculopathy in which structural changes caused by excessive vascular cell growth and inflammation play a major role.[2]

White blood cell (WBC) count and its subtypes have been studied as inflammatory markers for predicting adverse cardiovascular outcomes.[3,4] Of these

Received: April 11, 2013 *Accepted:* June 12, 2013

Correspondence: Dr. Abdulkadir Yıldız. Dicle Üniversitesi, Kalp Hastanesi, Diyarbakır, Turkey.
Tel: +90 412 - 248 80 01 e-mail: drakadiryildiz@gmail.com

© 2013 Turkish Society of Cardiology

subtypes, the neutrophil to lymphocyte ratio (N/L ratio), was found in several studies to be associated with severity of the disease and prognosis.[5,6]

To the best of our knowledge, N/L ratio has not been studied in patients with PAH. Thus, in this study, we sought to evaluate the association between N/L ratio and PAH.

PATIENTS AND METHODS

Study population

The study group consisted of 25 patients with PAH (16 females, mean age: 37.6±17.7 years), and the control group consisted of 25 healthy volunteers (15 females, mean age: 37.8±12.3 years). The study group included PAH patients who had undergone physical examination, echocardiography, and diagnostic cardiac catheterization. The etiologies were idiopathic in 14 patients and PAH associated with congenital heart disease in 11 patients. All PAH patients were on pulmonary-specific therapy. The control group underwent physical examination and echocardiography. PAH was defined as mean pulmonary arterial pressure (mPAP) ≥25 mmHg and pulmonary capillary wedge pressure ≤15 mmHg at rest detected by cardiac catheterization.[7] Patients were excluded from analysis if they were on warfarin treatment (n=2), or had cancer (n=1), systemic inflammatory disease (n=3), anemia (n=2), active infection (n=2), or left ventricular dysfunction (n=3). The institutional ethics committee approved the study and all patients gave their informed consent.

Biochemical measurements

Venous blood samples were drawn in the morning from the antecubital vein after a fasting period of 12 hours. Total and differential leukocyte counts were measured within 30 minutes of sampling by an automatic blood counter (Abbott Cell-Dyn 3700; Abbott Laboratory, Abbott Park, IL). Glucose, creatinine, and C-reactive protein (CRP) levels were assessed by standard methods.

Statistical analysis

Data were analyzed with the Statistical Package for the Social Sciences (SPSS) software version 16.0 for Windows (SPSS Inc, Chicago, IL). The Kolmogorov-Smirnov test was used to verify the normality of distribution of continuous variables. Continuous variables were defined as means ± standard deviation; categorical variables were given as percentages. The independent sample t test or the Mann-Whitney U test was used for the continuous variables and the chi-square test for categorical variables. Spearman test was used for correlation analysis. Statistical significance was defined as $p<0.05$. Receiver operating characteristic (ROC) curve analysis was used to determine the optimum cutoff levels of N/L ratio in association with PAH. Multivariate logistic regression analysis was performed to assess the independent predictors of PAH. All variables that were found significant in univariate analysis were included in the logistic regression model, and results are shown as an odds ratio (OR) with 95% confidence intervals (CIs).

Abbreviations:

CRP	*C-reactive protein*
IL	*Interleukin*
IPAH	*Idiopathic PAH*
MPV	*Mean platelet volume*
N/L	*Neutrophil to lymphocyte*
PAH	*Pulmonary arterial hypertension*
PDW	*Platelet distribution width*
RDW	*Red cell distribution width*
ROC	*Receiver operating characteristic*
SPSS	*Statistical Package for the Social Sciences*
WBC	*White blood cell*

RESULTS

Baseline demographic, biochemical and hemodynamic parameters of the study and control groups are shown in Table 1. There were no statistically significant differences between the two groups with respect to age, gender, systolic and diastolic blood pressures, and levels of glucose, creatinine, CRP, hemoglobin, WBC, and platelet count. Mean platelet volume (MPV) and platelet distribution width (PDW) were significantly higher among patients with PAH when compared with the control group (8.94±1.38 *vs.* 7.99±0.76 fl, p=0.005 and 18.14±1.23 *vs.* 17.4±0.56, p=0.005, respectively). Compared with the control group, red cell distribution width (RDW) and N/L ratio were significantly higher among patients with PAH (17.29±2.15 *vs.* 15.63±0.78, p=0.05 and 2.44±1.06 *vs.* 1.55±0.38, p=0.05, respectively; Fig. 1).

We analyzed the correlation between PAP and N/L ratio and RDW by Spearman test. According to the Spearman test, PAP was correlated with N/L ratio and RDW (r=0.293, p=0.046 and r=0.414, p=0.004, respectively). There was also a good correlation between N/L ratio and RDW (r=0.302, p=0.039).

After multivariate logistic regression analysis, only N/L ratio remained a significant predictor of PAH (OR: 5.472, 95% CI: 1.432-20.908, p=0.01;

Table 1. Comparison of baseline characteristics and laboratory and hemodynamic parameters

	PAH (n=25) Mean±SD	Control (n=22) Mean±SD	*p*
Age (years)	37.6±17.7	37.8±12.3	0.97
Sex (Male/Female)	9/16	7/15	0.76
Systolic blood pressure (mmHg)	112±11.64	112.05±9.84	0.98
Glucose (mg/dl)	94.32±8.73	91.59±5.53	0.21
Creatinine (mg/dl)	0.78±0.24	0.78±0.17	0.96
Total cholesterol (mg/dl)	162.83±31.06	189±37.13	0.06
Triglycerides (mg/dl)	129.58±54.90	149.53±92.70	0.51
High-density lipoprotein-cholesterol (mg/dl)	40.45±6.58	45.26±9.19	0.14
Low-density lipoprotein-cholesterol (mg/dl)	97.73±19.85	113.73±34.71	0.17
Hemoglobin (g/dl)	13.80±2.96	13.40±1.52	0.57
Red cell distribution width (%)	17.29±2.15	15.63±0.78	**0.001**
Platelet count (x10^9)	242.80±94.75	272.27±39.03	0.18
Mean platelet volume (fl)	8.94±1.38	7.99±0.76	**0.007**
Platelet distribution width (%)	18.14±1.23	17.4±0.56	0.013
White blood cell (x10^3 mg/dl)	8.02±1.75	8.29±2.19	0.64
Neutrophil (%)	58.66±14.86	54.16±5.81	0.19
Lymphocyte (%)	28.33±8.83	36.18±5.63	**0.001**
Neutrophil to lymphocyte ratio	2.44±1.06	1.55±0.38	**0.001**
C-reactive protein	0.52±0.21	0.51±0.19	0.81
Systolic pulmonary arterial pressure	78.04±31.00		
Mean pulmonary arterial pressure	46.03±18.12		

Data presented are mean values ± SD. Significance was set at p<0.05. PAH: Pulmonary arterial hypertension; SD: Standard deviation.

Table 2). By ROC analysis, a level of N/L ratio ≥1.65 predicted the presence of PAH with 72% sensitivity and 69% specificity (ROC area under curve: 0.767, 95% CI: 0.628-0.906, p=0.002; Fig. 2).

DISCUSSION

To our knowledge, N/L ratio has never been studied in PAH, and this is the first study showing the independent relationship between PAH and N/L ratio, which is a simple and reliable indicator of inflammation.

Regardless of the initial trigger, the elevated pulmonary vascular resistance in patients with PAH is primarily caused by endothelial dysfunction, remodeling, vasoconstriction, and thrombosis of small and medium pulmonary arteries.

Endothelial dysfunction is associated with a re-

Table 2. Multivariate logistic regression analysis to assess predictors of pulmonary arterial hypertension

	Odds ratio (95% CI)	*p*
Neutrophil to lymphocyte ratio	5.472 (1.432-20.908)	.013
Mean platelet volume	0.677 (0.190-2.406)	.546
Platelet distribution width	4.185 (0.810-21.620)	.088
Red cell distribution width	1.872 (0.944-3.712)	.072

CI: Confidence interval.

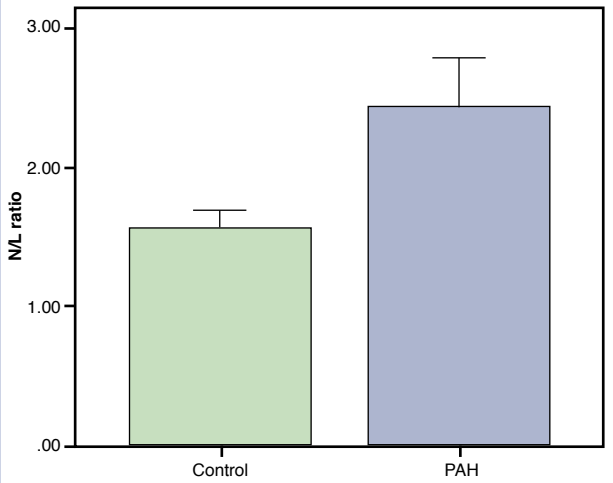

Figure 1. Neutrophil to lymphocyte ratio in patients with pulmonary arterial hypertension and the control group. N/L ratio: Neutrophil to lymphocyte ratio; PAH: Pulmonary arterial hypertension.

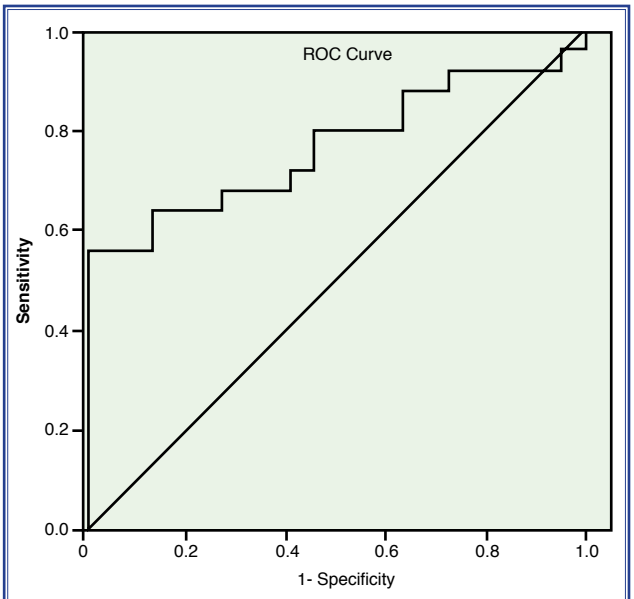

Figure 2. The receiver operating characteristic (ROC) curve analysis of neutrophil to lymphocyte ratio for predicting the presence of pulmonary arterial hypertension.

duction in endothelium-dependent vasodilatation and enhanced vasoconstriction. Adhesion and migration of circulating inflammatory cells occur at sites of endothelial damage, but the cascade of the signaling pathways resulting from such damage and the endothelial dysfunction associated with the inflammatory changes are not well understood in PAH.[8]

There is increasing evidence that inflammation and oxidative stress play a role in the pathophysiology of PAH with elevated levels of many cytokines and chemokines in affected patients.[9,10] Interleukin (IL)-6 has been linked to the development of pulmonary hypertension in experimental models.[11] Local inflammation and an activated complement system may affect neutrophils and platelets and contribute to plugging in the microvasculature and further vasoconstriction. The resulting vascular remodeling and proliferation cause a chronic elevation in pulmonary vascular resistance, right heart failure and eventually death.

White blood cell subtypes have an important role in modulating the inflammatory response in the PAH pathogenesis. Particularly, lymphocytopenia is a common finding in critical inflammatory states due to the increased lymphocyte apoptosis.[12] CD4+CD25+ cells are regulatory T lymphocytes (Treg) responsible for peripheral immune tolerance and are diminished in autoimmune diseases. There are conflicting data about which lymphocyte subgroup is affected in patients with PAH. Nicolls et al.[13] showed an association between PAH and decreased CD4+ T-cell compartment and a decreased CD4+/CD8+ ratio, similar to the autoimmune or connective tissue disorders. Because of the decreased or ineffective Treg cells, B-cell dysregulation and breakage of natural self-tolerance occur, resulting in increased autoantibodies and immune reaction to self tissues. In another study, Ulrich et al.[14] showed increased CD4+ regulatory and decreased CD8+ cytotoxic T cells in patients with idiopathic PAH (IPAH). They speculated that the increase in Treg cells in the blood of IPAH patients could be attributed to the necessity to suppress any self-reactive T cells for the inhibition of autoimmune disease development and that CD8+ T cells were exhausted, similar to a disease process in chronic viral infections.

Consistent with the literature, we found significantly less lymphocytes in PAH patients compared with controls. As N/L ratio reflects the balance between neutrophil and lymphocyte levels in the body, it is an indicator of systemic inflammation.[4,15] The present study showed that N/L ratio, a promising marker of inflammation, was increased in PAH. Moreover, a level of N/L ratio ≥1.65 predicted the presence of PAH with 72% sensitivity and 69% specificity. Compared to many other inflammatory markers, N/L ratio is inexpensive, widely available and routinely measured on admission; therefore, it adds no further cost.

Previous studies have demonstrated the relationship between N/L ratio and coronary artery disease severity, bare metal stent stenosis, and metabolic syndrome, etc.[5,6,16]

Although N/L ratio was significantly elevated in the PAH group, CRP levels did not differ between the PAH and control groups. This may be due to the exclusion of patients with increased CRP assuming a subclinical infection or the suppression of CRP with specific therapy.

Mean platelet volume and PDW are morphological parameters used in the assessment of platelet reactivity indirectly, and their association with PAH has been investigated in several studies.[17-19] In patients with PAH, systemic inflammation might cause platelet activation and increase in MPV values by stimulating megakaryocytes.[20]

Red cell distribution width quantifies the variability in the size of circulating red blood cells. Inflammation, dysfunctional erythropoiesis, iron deficiency, and oxidative stress are some of possible mechanisms of elevated RDW, which is associated with poor outcomes in various cardiovascular disorders, pulmonary emboli and pulmonary hypertension.[21-24] In our study, MPV, PDW and RDW tended to increase in the PAH group with respect to the control group. However, only N/L ratio remained statistically significant after multivariate analysis. N/L ratio was well correlated with RDW, supporting the hypothesis that RDW is a marker of underlying inflammation.

Our study has several limitations. This study represented a single center experience conducted on a small patient group due to the rarity of the disease. It provides no information regarding the cause or effect relationship between N/L ratio and PAH. Usage of a single blood sample will not anticipate the persistence of N/L ratio over time.

In conclusion, we showed that N/L ratio was significantly increased in patients with PAH compared with controls. These data may support previous trials reporting that hemostatic abnormalities may be involved directly in the pathogenesis of PAH, but this needs to be confirmed in larger randomized studies with more comprehensive assessment of hemostatic parameters. Understanding the exact role of the inflammatory pathway in the PAH pathogenesis may lead to new therapeutic approaches.

Conflict-of-interest issues regarding the authorship or article: None declared.

REFERENCES

1. Tuder RM, Abman SH, Braun T, Capron F, Stevens T, Thistlethwaite PA, et al. Development and pathology of pulmonary hypertension. J Am Coll Cardiol 2009;54:3-9.
2. Schermuly RT, Ghofrani HA, Wilkins MR, Grimminger F. Mechanisms of disease: pulmonary arterial hypertension. Nat Rev Cardiol 2011;8:443-55.
3. Gurm HS, Bhatt DL, Lincoff AM, Tcheng JE, Kereiakes DJ, Kleiman NS, et al. Impact of preprocedural white blood cell count on long term mortality after percutaneous coronary intervention: insights from the EPIC, EPILOG, and EPISTENT trials. Heart 2003;89:1200-4.
4. Horne BD, Anderson JL, John JM, Weaver A, Bair TL, Jensen KR, et al. Which white blood cell subtypes predict increased cardiovascular risk? J Am Coll Cardiol 2005;45:1638-43.
5. Kaya H, Ertas F, Islamoglu Y, Kaya Z, Atilgan ZA, Cil H, et al. Association Between Neutrophil to Lymphocyte Ratio and Severity of Coronary Artery Disease. Clin Appl Thromb Hemost 2013 May 7.
6. Turak O, Ozcan F, Isleyen A, Tok D, Sokmen E, Buyukkaya E, et al. Usefulness of the neutrophil-to-lymphocyte ratio to predict bare-metal stent restenosis. Am J Cardiol 2012;110:1405-10.
7. Badesch DB, Abman SH, Simonneau G, Rubin LJ, McLaughlin VV. Medical therapy for pulmonary arterial hypertension: updated ACCP evidence-based clinical practice guidelines. Chest 2007;131:1917-28.
8. Hall S, Brogan P, Haworth SG, Klein N. Contribution of inflammation to the pathology of idiopathic pulmonary arterial hypertension in children. Thorax 2009;64:778-83.
9. Bowers R, Cool C, Murphy RC, Tuder RM, Hopken MW, Flores SC, et al. Oxidative stress in severe pulmonary hypertension. Am J Respir Crit Care Med 2004;169:764-9.
10. DeMarco VG, Habibi J, Whaley-Connell AT, Schneider RI, Heller RL, Bosanquet JP, et al. Oxidative stress contributes to pulmonary hypertension in the transgenic (mRen2)27 rat. Am J Physiol Heart Circ Physiol 2008;294:H2659-68.
11. Steiner MK, Syrkina OL, Kolliputi N, Mark EJ, Hales CA, Waxman AB. Interleukin-6 overexpression induces pulmonary hypertension. Circ Res 2009;104:236-44.
12. Hotchkiss RS, Karl IE. The pathophysiology and treatment of sepsis. N Engl J Med 2003;348:138-50.
13. Nicolls MR, Taraseviciene-Stewart L, Rai PR, Badesch DB, Voelkel NF. Autoimmunity and pulmonary hypertension: a perspective. Eur Respir J 2005;26:1110-8.
14. Ulrich S, Nicolls MR, Taraseviciene L, Speich R, Voelkel N. Increased regulatory and decreased CD8+ cytotoxic T cells in the blood of patients with idiopathic pulmonary arterial hypertension. Respiration 2008;75:272-80.

15. Kalay N, Dogdu O, Koc F, Yarlioglues M, Ardic I, Akpek M, et al. Hematologic parameters and angiographic progression of coronary atherosclerosis. Angiology 2012;63:213-7.
16. Buyukkaya E, Karakas MF, Karakas E, Akçay AB, Kurt M, Tanboga IH, et al. Correlation of Neutrophil to Lymphocyte Ratio With the Presence and Severity of Metabolic Syndrome. Clin Appl Thromb Hemost 2012 Sep 18.
17. Can MM, Tanboğa IH, Demircan HC, Ozkan A, Koca F, Keleş N, et al. Enhanced hemostatic indices in patients with pulmonary arterial hypertension: an observational study. Thromb Res 2010;126:280-2.
18. Güvenc TS, Erer HB, Ilhan S, Zeren G, Ilhan E, Karakuş G, et al. Comparison of mean platelet volume values among different causes of pulmonary hypertension. Cardiol J 2012;19:180-7.
19. Arslan D, Cimen D, Guvenc O, Kaya F, Sert A, Oran B. Platelet distribution width and mean platelet volume in children with pulmonary arterial hypertension secondary to congenital heart disease with left-to-right shunt: new indices of severity? Pediatr Cardiol 2013;34:1013-6.
20. Soon E, Holmes AM, Treacy CM, Doughty NJ, Southgate L, Machado RD, et al. Elevated levels of inflammatory cytokines predict survival in idiopathic and familial pulmonary arterial hypertension. Circulation 2010;122:920-7.
21. Gul M, Uyarel H, Ergelen M, Karacimen D, Ugur M, Turer A, et al. The relationship between red blood cell distribution width and the clinical outcomes in non-ST elevation myocardial infarction and unstable angina pectoris: a 3-year follow-up. Coron Artery Dis 2012;23:330-6.
22. Zalawadiya SK, Zmily H, Farah J, Daifallah S, Ali O, Ghali JK. Red cell distribution width and mortality in predominantly African-American population with decompensated heart failure. J Card Fail 2011;17:292-8.
23. Ozsu S, Abul Y, Gunaydin S, Orem A, Ozlu T. Prognostic Value of Red Cell Distribution Width in Patients With Pulmonary Embolism. Clin Appl Thromb Hemost 2012 Nov 8.
24. Rhodes CJ, Wharton J, Howard LS, Gibbs JS, Wilkins MR. Red cell distribution width outperforms other potential circulating biomarkers in predicting survival in idiopathic pulmonary arterial hypertension. Heart 2011;97:1054-60.

Key words: Biological markers; heart failure; hypertension, pulmonary; N/L oranı; pulmonary artery; inflammation.

Anahtar sözcükler: Biyolojik belirteç; kalp yetersizliği; hipertansiyon, pulmoner; N/L oranı; pulmoner arter; enflamasyon.